University of Groningen

A kinetic and structural investigation of DNA-Based asymmetric catalysis using first-generation ligands

Rosati, Fiora; Boersma, Arnold J.; Klijn, Jaap E.; Meetsma, Auke; Feringa, Ben L.; Roelfes, Gerard

Published in:
 Chemistry

DOI:
[10.1002/chem.200900456](https://doi.org/10.1002/chem.200900456)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
 Publisher's PDF, also known as Version of record

Publication date:
 2009

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Rosati, F., Boersma, A. J., Klijn, J. E., Meetsma, A., Feringa, B. L., & Roelfes, G. (2009). A kinetic and structural investigation of DNA-Based asymmetric catalysis using first-generation ligands. *Chemistry*, 15(37), 9596-9605. <https://doi.org/10.1002/chem.200900456>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

A Kinetic and Structural Investigation of DNA-Based Asymmetric Catalysis Using First-Generation Ligands

Fiora Rosati, Arnold J. Boersma, Jaap E. Klijn, Auke Meetsma, Ben L. Feringa,* and Gerard Roelfes*[a]

Abstract: The recently developed concept of DNA-based asymmetric catalysis involves the transfer of chirality from the DNA double helix in reactions using a noncovalently bound catalyst. To date, two generations of DNA-based catalysts have been reported that differ in the design of the ligand for the metal. Herein we present a study of the first generation of DNA-based catalysts, which contain ligands comprising a metal-binding domain linked through a spacer to a 9-aminoacridine

moiety. Particular emphasis has been placed on determining the effect of DNA on the structure of the Cu^{II} complex and the catalyzed Diels–Alder reaction. The most important findings are that the role of DNA is limited to being a chiral scaffold; no rate acceleration was observed in the presence of

Keywords: asymmetric catalysis • Diels–Alder reactions • DNA • enantioselectivity • ligand effects

DNA. Furthermore, the optimal DNA sequence for obtaining high enantioselectivities proved to contain alternating GC nucleotides. Finally, DNA has been shown to interact with the Cu^{II} complex to give a chiral structure. Comparison with the second generation of DNA-based catalysts, which bear bipyridine-type ligands, revealed marked differences, which are believed to be related to the DNA microenvironment in which the catalyst resides and where the reaction takes place.

Introduction

The high activities and selectivities achieved by enzymes under mild conditions continue to be a source of inspiration for the design of new catalysts. A recent approach that has proven to be quite versatile is the creation of hybrid catalysts in which the catalytic power of transition-metal complexes is combined with the chiral architecture of biopolymers.^[1–5] This concept involves placing a metal complex in the chiral microenvironment provided by the biomolecular scaffold using covalent, supramolecular, or dative anchoring strategies. By using a protein scaffold, this method has resulted in a range of enantioselective artificial metalloenzymes that are capable of catalyzing transformations such as

transfer hydrogenation,^[6] hydrolysis,^[7] hydrogenation,^[8–10] epoxidation,^[11,12] sulfoxidation,^[13–16] Diels–Alder reactions,^[17,18] transamination,^[19] and allylic alkylation.^[20]

Polynucleotides such as RNA and DNA are arguably some of the most elegant chiral structures in nature and, hence, they are attractive scaffolds for the assembly of enantioselective hybrid catalysts. Prior to our studies it was demonstrated that the chirality of DNA can be transferred in a stoichiometric DNA-templated reaction.^[21–23] Furthermore, a DNAzyme capable of enantioselection of an RNA substrate^[24] and an enantioselective Diels–Alderase RNAzyme have been reported.^[25]

In our approach to DNA-based enantioselective catalysis,^[26–28] we have demonstrated that the chirality of DNA can be transferred directly to a metal-catalyzed reaction. The design involves the modular assembly of a DNA-based catalyst from natural duplex DNA, usually salmon testes DNA (st-DNA), and a copper complex of an achiral ligand that can bind DNA in a noncovalent fashion.^[26] As a result, the active Cu^{II} center is brought into the proximity of the chiral environment of the DNA double helix, allowing the transfer of chirality and the generation of reaction products with an excess in one of their enantiomers (Scheme 1).

To date, two generations of DNA-based catalysts have been developed that differ in the type of ligand used. In our

[a] F. Rosati, A. J. Boersma, Dr. J. E. Klijn, A. Meetsma, Prof. B. L. Feringa, Dr. G. Roelfes
Stratingh Institute for Chemistry, University of Groningen
Nijenborgh 4, 9747 AG Groningen (The Netherlands)
Fax: (+31) 50-363-4296
E-mail: j.g.roelfes@rug.nl
b.l.feringa@rug.nl

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/chem.200900456>.

Scheme 1. a) Schematic representation of the concept of DNA-based asymmetric Diels-Alder reaction; b) first (L1–L6) and second (dmbpy) generation of ligands used in DNA-based asymmetric catalysis.

first design, a metal-binding domain, that is, aminomethylpyridine, was linked through a short spacer to a DNA intercalator, that is, 9-aminoacridine. With this design enantiomeric excess (*ee*) values of up to 50% were obtained in Cu^{II} -catalyzed Diels-Alder reactions in water.^[26] The design of the ligand proved to have an important influence on the results of the catalysis. It was found that the ligand should preferably contain an arylmethyl group, that is, 3,5-dimethoxybenzyl or 1-naphthylmethyl, which suggests that π - π stacking interactions with the substrate are important for the observed enantioselective catalysis. Moreover, both enantiomers of the product were accessible by appropriate design of the ligand; the enantiomeric preference of the reaction was demonstrated to depend both on the nature of the arylmethyl substituent and the length of the spacer.

In an alternative design, the DNA-binding moiety was integrated into the metal-binding domain; hence, a spacer was no longer required. With this second generation of ligands, a dramatic increase in enantioselectivity was observed; up to 99% *ee* was obtained with 4,4'-dimethyl-2,2'-bipyridine (dmbpy) as the ligand.^[27,28] In these reactions it was found

that DNA is more than a chiral scaffold; rate accelerations of up to two orders of magnitude were observed in the presence of DNA. Moreover, both the rate acceleration and the enantioselectivity were found to be DNA-sequence dependent.^[29]

The scope of DNA-based catalysis has been extended beyond the Diels-Alder reaction; good-to-excellent enantioselectivities have been reported in the Michael addition reactions of malonates and nitromethane to yield α,β -unsaturated 2-acylimidazoles,^[30] Friedel-Crafts alkylations,^[31] fluorination reactions,^[32a] and epoxide hydrolysis.^[32b] Compared with the first generation of catalysts, however, the second generation of catalysts are less flexible with regard to which enantiomer of the product is obtained; the product resulting from attack of the diene or nucleophile on the *Si* face of the enone moiety is always obtained.

Recently, a variety of other approaches towards DNA-based catalysis have been reported, which feature a covalently attached catalytic moiety.^[33]

Herein we report the results of a study aimed at obtaining a better understanding of the mechanistic and structural aspects of the first generation of DNA-based catalysts, which comprise an acridine-based ligand. In particular the effects DNA has on the observed catalysis and on the structure of the catalyst will be discussed.

Results and Discussion

Kinetics: The effect of DNA on the reaction rate was investigated by studying the reaction kinetics. The mechanism by which a Lewis acid, that is, Cu^{II} , can be expected to affect the rate of the Diels-Alder reaction of azachalcone (1) with cyclopentadiene (2) in water is depicted in Scheme 2.^[34a] The first step in the cycle comprises a rapid and reversible coordination of the dienophile to the Cu^{II} ion, which results in the activation of the dienophile. This is followed by the irreversible Diels-Alder reaction. Finally, the product dissociates from the Lewis acid, regenerating the catalyst for another cycle of the reaction.

The overall rate of the reaction is determined by the equilibrium constant for the binding of the azachalcone (1) to Cu^{II} (K_a), the rate of the reaction of cyclopentadiene (2) with the Cu^{II} -bound azachalcone (k_{cat}), and the dissociation constant for the release of the product from the copper complex (K_d). In the kinetic measurements K_d did not influence the overall rate because a large excess of catalyst was used. Consequently, product inhibition can be excluded.

The apparent second-order rate constant (k_{app}) was determined from the initial decrease in the absorption of azachalcone (1) at various copper concentrations. From this, K_a and k_{cat} were determined by using the pseudophase model, as reported by Engberts and co-workers,^[34] using an iterative fitting approach. For practical reasons, the experiments were performed at a pH of 5.5 (MES buffer), which is different from the pH used in the catalytic experiments, that is, pH 6.5 (MOPS buffer). The use of a lower pH value result-

Scheme 2. Proposed catalytic cycle for the copper-catalyzed Diels–Alder reaction of **1** with **2**.

ed in only a slight decrease in the enantioselectivity of the catalytic experiments.^[35]

A comparison of the values of k_{app} obtained with ligands L1–L4 showed that the reactions were roughly equally fast,

Table 1. Dependence of kinetic data on DNA in the Cu–L catalyzed Diels–Alder reaction of **1** with **2** in water.^[a]

Entry	Ligand	k_{app} [$10^{-2} \text{ M}^{-1} \text{ s}^{-1}$] ^[b]	K_{a} [M^{-1}]	k_{cat} [$\text{M}^{-1} \text{ s}^{-1}$]
1	L1	3.1 ± 0.92	$1.8 \pm 0.74 \times 10^4$	0.111 ± 0.027
2	L1 ^[c]	2.6 ± 0.49	$8.2 \pm 0.97 \times 10^3$	0.100 ± 0.008
3	L2	3.50 ± 0.008	$1.0 \pm 0.40 \times 10^4$	0.218 ± 0.066
4	L2 ^[c]	1.5 ± 0.49	$1.2 \pm 0.45 \times 10^4$	0.057 ± 0.016
5	L3	4.0 ± 0.36	$2.3 \pm 1.40 \times 10^4$	0.113 ± 0.034
6	L3 ^[c]	1.50 ± 0.009	$2.5 \pm 0.97 \times 10^4$	0.046 ± 0.008
7	L4	3.7 ± 0.41	$1.9 \pm 0.92 \times 10^4$	0.124 ± 0.032
8	L4 ^[c]	3.4 ± 0.20	$1.2 \pm 0.58 \times 10^4$	0.064 ± 0.023

[a] $[\text{Cu}^{2+}\text{--L}] = 0.10\text{--}0.30 \text{ mM}$, 25.0°C , MES buffer (20 mM, pH 5.5); **1** = $6.0 \times 10^{-3} \text{ mM}$, **2** = $0.5\text{--}2.0 \text{ mM}$. [b] $[\text{Cu}^{2+}] = 0.058 \text{ mM}$. [c] In the presence of st-DNA.

regardless of the ligand (Table 1). In the presence of DNA a slight decrease in the reaction rate was observed in all cases.

A more detailed analysis revealed that the K_{a} values did not change significantly with the different ligands used. Hence, it can be concluded that the binding of the Lewis acid to the dienophile occurs with equal efficiency in the absence and presence of DNA. The lower overall reaction rates proved to be due to a lowering of the k_{cat} with ligands L2–L4. The observed differences are not large, but signifi-

cant. Notably, in the case of ligand L1 no significant decrease in k_{cat} was observed (Table 1, entries 1 and 2).

Attempts were made to determine the K_{a} values independently by a UV/Vis titration experiment^[29] to validate the values derived from the model used. However, these measurements were complicated by the strong absorption of the acridine chromophore in the wavelength region of interest. Therefore, K_{a} was determined for the Cu^{II} complex of a similar ligand (ligand L5, Scheme 1), which contains the same metal-binding domain but lacks the acridine moiety. By using this copper complex a K_{a} value of $(2.97 \pm 0.90) \times 10^4 \text{ M}^{-1}$ was found for the binding of azachalcone (**1**). This is similar to the values observed with the copper complexes of ligands L1–L4, which supports the accuracy of the values derived from the iterative fitting approach.

The results of the kinetic studies are in sharp contrast to those obtained with the second generation of catalysts: with Cu–dmpby, the presence of DNA resulted in a rate acceleration with an acceleration factor of 58.^[29] This suggests that the DNA-based catalysts, depending on the type of ligand, interact differently with the DNA, such that DNA has a distinct effect on the reaction rate. Most likely the reaction takes place in a different structural environment: In the case of the second-generation catalysts the reaction has been proposed to take place in the groove, which, for example, can provide favorable interactions with the activated complex.^[29] In the case of the first-generation catalysts, as a result of the design, the reaction is proposed to occur further away from the DNA groove. This is consistent with the observed decrease in *ee* when using ligands with a longer spacer.^[26] Therefore, the reaction is more comparable to that taking place in bulk solution, that is, in the absence of DNA. Hence, only minor effects on the reactivity are observed. Thus, in the present case, DNA can be considered to act exclusively as the chiral scaffold.

DNA-sequence dependence: The structure of the second coordination sphere provided by DNA has been demonstrated to have a strong effect on the enantioselectivity of second-generation DNA-based catalytic Diels–Alder^[29] and Friedel–Crafts reactions.^[31] st-DNA is a heterogeneous polymer and its sequence can be considered to be random. Loadings of one complex per six base pairs were used in the catalytic

experiments. Therefore, the DNA-based catalyst is a mixture of copper–ligand complexes located in different chiral microenvironments. Consequently, the observed enantioselectivity most likely is the (weighted) average of all the contributing DNA sequences.

A series of different DNAs were tested to establish the effect of the chiral microenvironment provided by the DNA on the Cu–L1-catalyzed Diels–Alder reaction. DNA from an alternative natural source, that is, calf thymus DNA (ct-DNA), resulted in an *ee* comparable to that found with st-DNA (Table 2, entry 2). Next, a series of synthetic self-com-

further truncation to an octamer led to a significantly lower *ee* (Table 2, entry 7). This is possibly the result of the increased chance that the copper complex is located near one of the termini, where the influence of the chirality of the DNA is smaller.

It was interesting to note that the results obtained with the d(GC)₆ sequence are dependent on the presence of salt; in the absence of NaCl a significantly lower *ee* was found (Table 2, entries 7 and 8). Surprisingly, the enantioselectivity could be restored by interchanging the order of the G and C nucleobases; with d(CG)₆ an *ee* of 60% was obtained. The origins of these effects are, at present, not well understood, but are most likely related to small differences in the structure of the DNA.

The high enantioselectivities obtained with alternating GC sequences can tentatively be explained by the acridine moieties having an increased affinity for GC-rich regions.^[36–38] Alternatively, the coordination sphere provided by GC-rich regions may provide the most favorable environment for the catalyzed reaction.

Substrates: An investigation of the substrate scope previously revealed that dienophiles able to chelate copper in a bidentate fashion is a general requirement for both catalysis and enantioselectivity.^[29] The general design is amenable to variations in the metal-binding domain, however, an N,O donor set is required.

For example, the pyridine moiety can be replaced by an *N*-methylimidazole moiety.^[29] The role of the pyridine in the interaction with DNA was probed by introducing a methyl substituent onto the pyridyl ring and systematically varying its position. It was found that, depending on the ligand used, this affected the conversion significantly (Table 3). However, no clear pattern could be discerned. The *endo* isomer of the Diels–Alder product was always favored, albeit with ligand L1 a significantly lower *endo/exo* ratio was always observed (Table 3, entries 9–12). The magnitude of the *ee* did not change to a major extent for **4–6** compared with the unsubstituted azachalcone (Table 3).

Which enantiomer is formed in excess depends on the ligand used. Interestingly, ligand L4 always gives the opposite enantiomer of the Diels–Alder product compared with ligands L1–L3, except with the substrate **6**, which has the methyl substituent at the 3-position of the pyridyl ring. This particular behavior can be explained tentatively on the basis that the azachalcone substrate can adopt either a *cisoid* or *transoid* conformation. In the case of **6**, the dienophile is forced to react in the *cisoid* conformation because the *transoid* form is unfavored for steric reasons (Scheme 3).^[39] Most likely this is the reason why with **6** the same enantiomer is obtained in excess with all ligands.

However, with substrates **4** and **5** this limitation does not exist and we propose that with the ligand L4 a different substrate-bound complex is formed preferentially,^[39] that is, with the dienophile in the *transoid* conformation (Scheme 3). Assuming that the conformation of the Cu-bound dienophile is an important determinant for the enan-

Table 2. Dependence of enantioselectivity on the DNA sequence using ligand L1.^[a]

Entry	DNA sequence	Conversion [%]	<i>ee</i> ^[b] [%]
1	st-DNA	100	37
2	ct-DNA	100	35
3	d(ATATATATATAT) ₂	100	9
4	d(A ₆ T ₆) ₂	100	6
5	Poly(dA–dT)(dA–dT)	100	6
6	Poly(dG–dC)(dG–dC)	100	62
7	d(GCGCGCGC) ₂ ^[c]	100	27
8	d(GCGCGCGCGCGC) ₂ ^[c]	100	54
9	d(GCGCGCGCGCGC) ₂	100	35
10	d(CGCGCGCGCGCG) ₂	90	60
11	d(G ₆ C ₆) ₂	100	8
12	d(CGCGGCGCGCGCG) ₂	100	35
13	d(CGCGCCGCGCGCC) ₂	97	26
14	d(CGCGGGCCCGCG) ₂	97	25
15	d(TCAGGGCCCTGA) ₂	100	10
16	d(TCGCGATCGCGA) ₂	84	10
17	d(TCGGGTACCCGA) ₂	84	16
18	d(TCGCGTACGCGA) ₂	100	19
19	d(CGCGATATCGCG) ₂	100	8
20	d(CGCGTATACGCG) ₂	100	28
21	d(GGGCAATTGCC) ₂	100	3
22	d(CGCGAATTCGCG) ₂	92	15
23	d(CGCAAATTTGCG) ₂	100	16
24	d(GCAAAATTTTGC) ₂	100	4
25	d(GCATGCATCGTACGTA) ₂	100	9
26	d(GACTGACTAGTCAGTC)	100	34

[a] Conditions: see the Experimental Section. [b] *ee* values refer to the (+) enantiomer in all cases and are the average of two experiments. They are reproducible to within 2%. [c] 100 mM NaCl.

plementary oligonucleotides of defined sequence were tested. The sequences were selected to cover a broad range of the available sequence space. From the data acquired (Table 2), a general pattern can be discerned. First, sequences that are rich in AT base pairs generally give rise to very poor enantioselectivities (Table 2, entries 3–5). Furthermore, in sharp contrast to the results obtained with Cu–dmbpy,^[29] sequences containing stretches of guanines give *ees* that are significantly lower than those obtained with st-DNA (Table 2, entries 14–15, 17, and 21). The best results were obtained with sequences rich in GC base pairs, in particular with alternating GC sequences (Table 2, entries 6–10); up to 62% *ee* was obtained with poly(GC) (Table 2, entry 6), which is significantly higher than the value obtained with st-DNA. A GC dodecamer gave similar *ee* values, however,

Table 3. Substrate variations in the Diels–Alder reaction with cyclopentadiene.^[a]

Entry	Ligand	Dienophile	Conversion ^[b] [%]	endo/ exo	endo ee [%] ^[c]
1	L1		55	88:12	–46
2	L2	4	75	99:1	–40
3	L3	4	100	97:3	–59
4	L4	4	> 80	96:4	45
5	L1		85	90:10	–34
6	L2	5	72	98:2	–40
7	L3	5	46	99:1	–46
8	L4	5	50	99:1	45
9	L1		31	88:12	–33
10	L2	6	68	78:22	–54
11	L3	6	29	73:27	–53
12	L4	6	100	95:5	–54

[a] Conditions: all experiments were carried out with the following reagents: $[\text{Cu}(\text{L})(\text{NO}_3)_2] = 0.3 \text{ mM}$, $\text{st-DNA} = 1.3 \text{ mg mL}^{-1}$, $[\mathbf{4-6}] = 1 \text{ mM}$, and $[\mathbf{2}] = 15 \text{ mM}$ in MOPS buffer (20 mM, pH 6.5) for 3 days at 5 °C. [b] Determined by ^1H NMR spectroscopy. [c] +/– refers to the order of elution of the two enantiomers, first and second, respectively (see the Experimental Section). The *endo/exo* ratio, *ee*, and conversion data are reproducible to within 3%.

Scheme 3. Possible reactive conformations of the unsubstituted dienophile: a) *cisoid*, b) *transoid*.

tiofacial selectivity of the reaction, this results in the formation of the opposite enantiomer of the product formed with ligands L1–L3 in which the substrate is most likely bound to the Cu^{II} center in the *cisoid* conformation.

Crystal structure: The complexation of L1 with $[\text{Cu}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}]$ in CH_3CN in the presence of HClO_4 (1 equiv) resulted in the formation of dark-green crystals, of which the crystal structure was determined. The identification of the atoms and the configuration of the cation moieties as well as the packing of the molecules in the cell are shown in Figure 1. Each asymmetric unit contains 11 moieties consisting of two cations of mononuclear copper com-

Figure 1. ORTEP diagram of the Cu–L1 complexes 1 and 2 in the unit cell. Bond lengths: Cu–N_{eq} : 1.96–2.06 Å; Cu–N_{ax} : 2.24 Å.

plexes, six perchlorate anions, and three acetonitrile solvent molecules.

The asymmetric unit contains two chemically distinct mononuclear copper complexes. The structure of cation complex 1 (Figure 1, top) shows a mononuclear Cu^{II} center in a square pyramidal geometry. The ligand is bound in the equatorial plane and coordinates to the cation through the two nitrogen atoms of the aminomethylpyridine moiety. The Cu–N bond lengths range from 1.96 to 2.06 Å, which is characteristic for Cu^{II} complexes.^[40,41] The remaining three coordination sites are occupied by acetonitrile molecules, which was the solvent for crystallization. The structure of the cation complex 2 (Figure 1, bottom) is very similar, except for the fact that the three remaining coordination sites are occupied by two acetonitrile molecules and one water molecule. The latter is bound in the equatorial plane *trans* to the tertiary amine of the ligand. It is at these equatorial sites that the substrate is proposed to bind through the pyridyl nitrogen and carbonyl oxygen, thereby displacing the solvent molecules. An interesting aspect of this structure is that the acridine moiety of complex 1 lies in a parallel orientation above the dimethoxybenzyl moiety of complex 2, which in turn lies above the pyridyl moiety of complex 2. The distance between the planes of the aromatic rings is approximately 3.5 Å, which is characteristic of π – π stacking interactions.^[42,43]

The π – π stacking interactions are considered to be important for achieving enantioselectivity in the aqueous Diels–Alder reaction of azachalcone with cyclopentadiene.^[26] The present structure most likely does not reflect the structure of the complex in aqueous solution, but it does demonstrate the propensity of the ligand for π – π stacking interactions.

Circular dichroism (CD) spectroscopy: Because of the different behavior of the copper complexes of ligands L1 and L4 in catalysis, a CD study of the DNA–copper–ligand complexes was performed to determine whether this difference could be assigned to a different interaction with the DNA.

9-Aminoacridine is achiral, but upon interaction with DNA, which provides a chiral environment, an induced CD is observed.^[44] Signals from DNA are found in the region below 300 nm. However, in the presence of 9-aminoacridine, the CD spectrum shows characteristic absorption bands around 340 and 400 nm (Figure 2a).^[44,45]

Figure 2. a) CD spectra of copper–DNA–Lx: L1 (.....), L2 (---), L3 (—), and L4 (----) complexes; b) comparison of the complexes of ligands L1 (—), L3 (----), and L6 (.....).

For the ligands with a propyl spacer, that is, L2 and L4 (Scheme 1), a spectrum similar to 9-aminoacridine was observed with characteristic absorption bands around 330 and 400 nm (Figure 2a), which correspond to two (π – π^*) electronic transitions polarized along the short and long axes of the acridine molecule, respectively.^[45]

By using a ligand with a shorter spacer, that is, ligands L1 and L3, a different pattern was observed. An increase in the intensity of the absorption at around 400 nm was observed as well as an exciton coupling at 330 nm, which suggests a chiral interaction between the acridine moiety and one of the two possible chromophores on the ligand: The pyridyl ring or the aromatic substituent of the ligand, that is, the 1-naphthylmethyl or 3,5-dimethoxybenzyl moiety. For the ligands with a longer spacer, such as ligands L2 and L4, these chromophores are apparently too far from the DNA cavity to have a defined orientation with respect to the acridine.

Then, to identify which chromophore is involved in this interaction, a new design of ligand was employed in which the arylmethyl moiety on the nitrogen atom was replaced with an ethyl group (L6).^[35] By using the copper complex of this ligand with DNA, the exciton coupling was still observed (Figure 2b). From this it can be concluded that the aromatic side-chain in ligands L2 and L4 are not involved in the interaction that gives rise to the exciton coupling. Most likely the pyridyl chromophore interacts with the acridine, possibly as a result of partial intercalation, which suggests that it is located closer to the DNA groove. Importantly, the behavior observed by CD spectroscopy with ligands L1–L4 does not correlate with the enantiomeric outcome of the reaction; apparently the chiral structure of the complex is only present in the resting state of the catalyst.

Comparison with the second generation of DNA-based catalysts: Comparison between the two generations of DNA-based catalysts reveals marked differences, both with regard to enantioselectivity and the role DNA plays in the catalytic reactions.

- 1) Compared with the second-generation catalysts, which give rise to up to 99% *ee* in the Diels–Alder reaction, the *ee* values obtained with the first generation of DNA-based catalysts are modest.
- 2) Both enantiomers of the Diels–Alder product are accessible by using the first generation of catalysts, whereas with the complexes formed between DNA and the bipyridine (bpy)-based ligands, the enantiomer resulting from the approach of the cyclopentadiene at the *Si* face of the enone moiety is always favored.
- 3) DNA plays a different role in the catalysis depending on the ligand used. In combination with the copper complex of the 4,4'-dimethylbipyridine ligand, the presence of DNA results in a significant rate acceleration of up to two orders of magnitude. In contrast, with the first generation of ligands, there is a slight decrease in the reaction rate in the presence of DNA.
- 4) The first and second generation of catalysts also have different requirements regarding the DNA sequence and, hence, structure, with the acridine-based catalysts the best results are obtained with alternating GC sequences, whereas with the bpy-based catalysts the presence of guanine tracts is especially beneficial.

These differences can be rationalized by considering that the catalyzed reaction can take place in different microenvironments depending on the type of ligand used. The general design of the catalysts comprises a metal-binding domain and a DNA-binding domain to anchor the catalyst to the DNA. In the second generation of catalysts, which comprises planar, symmetric polyaromatic molecules, the DNA binding moiety is integrated into the metal binding site. Therefore, the catalytic metal center is most likely very close to the DNA helix and the distance from the DNA decreases on going from larger, for example, dipyrro[3,2-*a*:2',3'-*c*]phenazine (dppz), to smaller polyaromatic moieties such as bpy and dmbpy. It has been proposed that with the catalysts based on the bpy-type ligands, the Diels–Alder reaction takes place in the DNA groove, which provides a chiral environment that is potentially structurally compatible with the transition state of the reaction.^[29] The resulting stabilization of the transition state would explain the observed 58-fold rate enhancement and the high enantioselectivity that was found with Cu–dmbpy. This requires a specific structure of the DNA groove, as demonstrated by the strong DNA-sequence dependence of the enantioselectivity of the reaction, which was shown to be positively affected by sequences containing guanine tracts, which give rise to B-DNA, but with A-DNA characteristics.

In the first generation of ligands, the DNA binding moiety, that is, 9-aminoacridine, is linked to a metal-binding domain through a spacer. Hence, the catalyzed reaction is in this case expected to take place at the edge of the DNA groove and, therefore, will more resemble the reaction as it occurs in solution in the absence of DNA. As a result only small differences in reaction rate are observed between the reactions with and without DNA. Furthermore, the enantioselectivity does not originate from the structural compatibility of the DNA groove with one enantiomer of the transition state, but most likely by the shielding of one face of the enone by the arylmethyl side-chain of the ligand, as has also been suggested for the enantioselective Diels–Alder reaction catalyzed by copper–amino acid complexes.^[34] The interaction of the ligand with the substrate has been proposed to occur by π – π stacking interactions. This hypothesis implies that, in contrast to the bpy-based catalysts, with the first generation of DNA-based catalysts the chirality is not transferred directly from the DNA to the catalyzed reaction. Instead, the chirality is transferred in two steps; the DNA forces a chiral conformation on the bound copper complex, which is in turn translated into enantioselective bond formation in the catalyzed Diels–Alder reaction. Indeed, the induced CD effects, in particular the observed exciton coupling, demonstrate that the DNA can enforce a chiral structure on the copper complex, even though the observed induced CD effect proved to be unrelated to the enantiomeric preference of the reaction. Based on the results obtained by varying the substrates, we hypothesize that an important aspect in determining which enantiomer is formed in excess is whether the substrate binds in the *cisoid* or *transoid* conformation. This is in turn most likely determined by a com-

bination of the structure of the ligand, which is supported by the fact that both enantiomers of the product can be accessed depending on the design of the ligand, and the interaction of the DNA with the substrate-bound complex.

Conclusions

A detailed study of the first generation of DNA-based catalysts in copper-catalyzed Diels–Alder reactions in water was performed with a particular focus on establishing the role of DNA in catalyzed enantioselective Diels–Alder reactions. A comparison with the second generation of DNA-based catalysts, which involves bpy-type ligands, revealed marked differences, which are most likely the result of different microenvironments for the catalyzed reactions. These findings underline the fact that the design of the ligand is an important aspect in the development of hybrid catalysts: Depending on the ligand used, the catalyzed reactions can take place in very different microenvironments, which results in different reaction rates and enantioselectivities.

Experimental Section

General: st-DNA was obtained from Sigma, pAT and pGC were obtained from Amersham. Synthetic oligonucleotides were obtained from BioTez (Germany). Cyclopentadiene was freshly prepared from its dimer prior to use. Azachalcone dienophiles and acridine-based ligands (L1–L4) were prepared by following published procedures.^[35] For kinetic studies st-DNA was dialyzed extensively against MES buffer (20 mM, pH 5.5) prior to use. ¹H and ¹³C NMR spectra were recorded on a Varian 400 (400 MHz) spectrometer in CDCl₃. Chemical shifts (δ) are given in ppm using residual solvent as the internal standard ($\delta_{\text{C}} = 77.0$ ppm and $\delta_{\text{H}} = 7.26$ ppm for CDCl₃).

Physical methods—General: Circular Dichroism spectra were measured on a JASCO J-715 spectropolarimeter equipped with a temperature control attachment. The UV/Vis spectra were measured on JASCO V-560 and JASCO V-570 spectrophotometers. The *ees* were determined by HPLC analysis performed on a Shimadzu 10AD-VP system equipped with a Daicel ODH column (*n*-heptane/*i*PrOH, 98:2, 0.5 mL min^{−1}) or a Daicel OD column (*n*-heptane/*i*PrOH, 98:2, 1 mL min^{−1}).

Representative procedure for the [Cu(L)(NO₃)₂]/DNA-catalyzed Diels–Alder cycloaddition reaction: A solution of st-DNA (10 mL of a 2 mg mL^{−1} solution of st-DNA dissolved in 30 mM MOPS buffer, pH 6.5, and prepared 24 h in advance) was added to a catalyst solution of [Cu(L)(NO₃)₂] (0.9 mM of Cu and 1.17 mM of ligand in a total volume of 5 mL) to a final concentration of 1.3 mg mL^{−1}. An aliquot of a stock solution of dienophile azachalcone in CH₃CN (0.5 M, 30 μ L) was added to obtain a final concentration of 1 mM and the mixture was subsequently cooled to 5 °C. The reaction was started by the addition of cyclopentadiene (20 μ L, 0.016 M final concentration) and allowed to continue for 3 d at 5 °C, while mixing continuously. The product was isolated by extraction with Et₂O. After drying (Na₂SO₄) and removal of the solvent, the crude product was analyzed by ¹H NMR spectroscopy and HPLC.

Dissolution of synthetic oligonucleotides: The synthetic oligonucleotides were obtained from BioTez. The lyophilized powders were dissolved in MOPS buffer (20 mM, pH 6.5) and the solution was heated to 94 °C, slowly cooled to 5 °C, and left for 2 h at 5 °C prior to use. The concentration was determined by UV/Vis spectrophotometry at 25 °C.

Reactions in the presence of synthetic oligonucleotides: A 2 mL Eppendorf container was loaded with oligomer solution (400 μ L, 2 mg mL^{−1}) in buffer (20 mM MOPS, pH 6.5), [CuL1(NO₃)₂] complex (200 μ L, 0.3 mM),

and a stock solution of azachalcone (**1**; 2.4 μL , 3.1 mg/60 μL CH_3CN , 0.024 mM) at 5°C. Freshly distilled cyclopentadiene (**2**; 0.8 μL , 0.016 M final concentration) was added to this mixture through a microsyringe. The reaction mixture was mixed by continuous inversion for 3 d at 5°C. After extraction with Et_2O , drying with Na_2SO_4 , and concentration under reduced pressure, the products were analyzed by HPLC.

Determination of k_{app} : The procedure to determine k_{app} , K_{a} , and k_{cat} were adapted from the procedure of Engberts and co-workers.^[34] A fresh solution of azachalcone (**1**; 2.0 μL , 0.95 mM in CH_3CN) was added to the appropriate catalyst in buffer (20 mM MES, pH 5.5) in a quartz cuvette. After the absorption had stabilized, a freshly prepared cyclopentadiene solution in CH_3CN (1–10 μL , 0.5 M) was added, with a final concentration 0.5–2.0 mM in the reaction mixture. The cuvette was closed immediately to prevent evaporation of cyclopentadiene and sealed. The reaction was monitored at the appropriate temperature at 326 nm on a JASCO V-560 or JASCO V-570 spectrophotometer. The decrease in absorption of **1** was followed for the first 15% of the reaction, and Equation (1) was used to calculate k_{app} , in which ε_1 and ε_3 are the extinction coefficients of dienophile **1** and product **3**, respectively, and d is the path length of the cuvette. The observed rate constants were determined at different concentrations of cyclopentadiene **2** and the value of k_{app} was extracted from the slope of the resulting plot.

$$k_{\text{app}} = \frac{dA_1}{dt} \frac{1}{d(\varepsilon_1 - \varepsilon_3)[\mathbf{1}]_0[\mathbf{2}]_0} \quad (1)$$

Determination of K_{a} and k_{cat} : The rate of the reaction of the dienophile with the diene can be described by the sum of the rate of the catalyzed and uncatalyzed reactions [Eq. (2)].

$$\frac{d[\text{aza}]}{dt} = k_{\text{uncat}}[\text{Cp}]_{\text{tot}}[\text{aza}]_{\text{f}} + k_{\text{cat}}[\text{Cp}]_{\text{tot}}[\text{aza}]_{\text{b}} \quad (2)$$

In this equation k_{uncat} and k_{cat} are the uncatalyzed and catalyzed rate constants, respectively. The dienophile is either bound to the catalyst (Lewis acid) or free in solution, whereas the diene is distributed homogeneously through the solution. The concentrations of diene and dienophile are given by $[\text{Cp}]_{\text{tot}}$, $[\text{aza}]_{\text{tot}}$, $[\text{aza}]_{\text{f}}$, and $[\text{aza}]_{\text{b}}$, which represent the total concentration of the diene, the total concentration of the dienophile, the concentration of the free dienophile, and the concentration of the bound dienophile, respectively. The total concentration of the dienophile is given by the sum of the free and bound concentrations. Considering that at most 10% of the catalyst M^{n+} is bound to the dienophile (see below) instead of using the concentration of the free catalyst ($[\text{M}^{n+}]_{\text{f}}$), the total concentration of the catalyst ($[\text{M}^{n+}]_{\text{tot}}$) can be used to calculate the binding constant (K_{a}) of the dienophile to the catalyst [Eq. (3)]. From this, the mole fraction of bound dienophile (X_{b}) can be calculated [Eq. (4)].

$$K_{\text{a}} = \frac{[\text{aza}]_{\text{b}}}{[\text{aza}]_{\text{f}}[\text{M}^{n+}]_{\text{tot}}} \quad (3)$$

$$X_{\text{b}} = \frac{K_{\text{a}}[\text{M}^{n+}]_{\text{tot}}}{1 + K_{\text{a}}[\text{M}^{n+}]_{\text{tot}}} \quad (4)$$

Substitution of Equation (3) into Equation (2) leads to Equation (5).

$$\frac{d[\text{aza}]_{\text{tot}}}{dt} = k_{\text{uncat}}[\text{Cp}]_{\text{tot}}[\text{aza}]_{\text{f}} + k_{\text{cat}}[\text{aza}]_{\text{f}} + k_{\text{cat}}[\text{Cp}]_{\text{tot}} \frac{K_{\text{a}}[\text{M}^{n+}]_{\text{tot}}}{1 + K_{\text{a}}[\text{M}^{n+}]_{\text{tot}}} \quad (5)$$

Under pseudo first-order conditions (excess diene) the observed apparent rate constant ($k_{\text{obs,app}}$) can be obtained from the observed rate constant (k_{obs}) and the concentration of the diene [Eq. (6)].

$$k_{\text{obs,app}} = \frac{k_{\text{obs}}}{[\text{Cp}]_{\text{tot}}} \quad (6)$$

The apparent rate constant (k_{app}) can then be calculated after correcting for incomplete binding of the dienophile to the catalyst [Eq. (7)].

$$k_{\text{app}} = \frac{k_{\text{obs,app}}}{X_{\text{b}}} \quad (7)$$

Because the decrease in the dienophile concentration as a function of time is given by Equation (8) and because the conditions are such that the uncatalyzed reaction is negligible, Equations (5) and (8) can be combined to give Equation (9).

$$\frac{d[\text{aza}]_{\text{tot}}}{dt} = k_{\text{app}}[\text{aza}]_{\text{tot}}[\text{Cp}]_{\text{tot}} \quad (8)$$

$$k_{\text{app}} = k_{\text{cat}} \frac{K_{\text{a}}[\text{M}^{n+}]_{\text{tot}}}{1 + K_{\text{a}}[\text{M}^{n+}]_{\text{tot}}} \quad (9)$$

Equation (9) can be rewritten as Equation (10) so that a straight line is obtained by plotting $1/k_{\text{app}}$ versus $1/[\text{M}^{n+}]$. From the values of the slope and the intercept, values of K_{a} and k_{cat} can be obtained. Because k_{app} can only be calculated if K_{a} is known the data has to be fitted iteratively until the value of K_{a} inserted into Equation (4) converges to the value obtained from the ratio of the intercept to the slope.

$$\frac{1}{k_{\text{app}}} = \frac{1}{k_{\text{cat}}K_{\text{a}}} \frac{1}{[\text{M}^{n+}]_{\text{tot}}} + \frac{1}{k_{\text{cat}}} \quad (10)$$

Determination K_{a} :^[34] The K_{a} values were determined also independently by titration experiments using ligand L5 which contains the same binding site as the other ligands tested, but depleted of the acridine moiety. The catalyst solution without DNA was prepared as previously described in the literature.

Crystal structure determination: Data were collected on a Bruker SMART APEX CCD diffractometer (Platform with full three-circle goniometer). The diffractometer was equipped with a 4K CCD detector set 60.0 mm from the crystal. The crystal was cooled to 100 K using the Bruker KRYOFLEX low-temperature device. Intensity measurements were performed using graphite monochromated MoK_{α} radiation from a sealed ceramic diffraction tube (Siemens). Generator settings were 50 kV/40 mA. SMART was used for preliminary determination of the unit cell constants and data collection control. Data integration and global cell refinement was performed with the program SAINT. Intensity data were corrected for Lorentz and polarization effects, scale variation, for decay and absorption: a multi-scan absorption correction was applied, based on the intensities of symmetry-related reflections measured at different angular settings (SADABS), and reduced to F_o^2 . The program suite SHELXTL was used for space group determination (XPRED). The structure was solved by Patterson methods and extension of the model was accomplished by direct methods applied to difference structure factors using the program DIRDIF. Details of the data collection and refinement are given in Table 4.

CCDC-719143 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Table 4. Crystal data and details of the structure determination.

formula	$C_{36}H_{40}CuN_7O_2^{3+} \cdot [C_{34}H_{39}CuN_6O_3]^{3-} \cdot 6[ClO_4]^- \cdot 3(C_2H_3N)$
formula weight [g mol ⁻¹]	2029.43
crystal system	triclinic
space group (no.)	$P\bar{1}$ (2)
<i>a</i> [Å]	13.806(1)
<i>b</i> [Å]	16.682(2)
<i>c</i> [Å]	19.765(2)
α [°]	100.922(2)
β [°]	90.722(2)
γ [°]	99.422(2)
<i>V</i> [Å ³]	4404.9(8)
θ range unit cell: min.–max. [°], reflections	2.52–27.38, 7360
<i>Z</i>	2
ρ_{calcd} [g cm ⁻³]	1.530
<i>F</i> (000)	2096
μ (MoK α) [cm ⁻¹]	7.54
color, habit	green, platelet
approx. crystal dimensions [mm]	0.33 × 0.20 × 0.08
λ (MoK α) [Å]	0.71073
<i>T</i> [K]	100(1)
θ range: min., max. [°]	2.23, 25.03
total data	31406
unique data	15289
data with criterion: $F_o \geq 4.0\sigma(F_o^2)$	8551
$R_{\text{int}} = \Sigma [F_o^2 - F_g^2(\text{mean})] / \Sigma [F_o^2]$	0.0685
$R_{\text{sig}} = \Sigma \sigma(F_o^2) / \sigma[F_o^2]$	0.1354
number of reflections	15289
number of refined parameters	1153
final agreement factors:	
$wR(F^2) = \{\Sigma [w(F_o^2 - F_c^2)^2] / \Sigma [w(F_o^2)^2]\}^{1/2}$	0.1735
$R(F) = \Sigma (F_o - F_c) / \Sigma F_o $ (For $F_o > 4.0\sigma(F_o)$)	0.0690
$\text{GooF} = S = \{\Sigma [w(F_o^2 - F_c^2)^2] / (n - p)\}^{1/2}$	0.958

Acknowledgements

Financial support from ERA-Chemistry, the Netherlands Organisation for Scientific Research (NWO-CW), and NRSC-Catalysis is gratefully acknowledged.

- a) J. Steinreiber, T. R. Ward, *Coord. Chem. Rev.* **2008**, 252, 751–766; b) G. Roelfes, *Mol. BioSyst.* **2007**, 3, 126–135; c) R. Krämer, *Angew. Chem.* **2006**, 118, 872–874; *Angew. Chem. Int. Ed.* **2006**, 45, 858–860.
- M. D. Mihovilovic, *J. Chem. Technol. Biotechnol.* **2007**, 82, 1067–1071.
- Y. Lu, *Curr. Opin. Chem. Biol.* **2005**, 9, 118–126.
- D. F. Qi, C. M. Tann, D. Haring, M. D. Distefano, *Chem. Rev.* **2001**, 101, 3081–3111.
- M. T. Reetz, *Proc. Natl. Acad. Sci. USA* **2004**, 101, 5716–5722.
- a) C. Letondor, A. Pordea, N. Humbert, A. Ivanova, S. Mazurek, M. Novic, T. R. Ward, *J. Am. Chem. Soc.* **2006**, 128, 8320–8328; b) C. Letondor, N. Humbert, T. R. Ward, *Proc. Natl. Acad. Sci. USA* **2005**, 102, 4683–4687.
- G. Klein, N. Humbert, J. Gradinaru, A. Ivanova, F. Gilardoni, U. E. Rusbandi, T. R. Ward, *Angew. Chem.* **2005**, 117, 7942–7945; *Angew. Chem. Int. Ed.* **2005**, 44, 7764–7767.
- M. T. Reetz, J. J. P. Peyralans, A. Maichele, Y. Fu, M. Maywald, *Chem. Commun.* **2006**, 4318–4320.
- R. R. Davies, M. D. Distefano, *J. Am. Chem. Soc.* **1997**, 119, 11643–11652.
- a) J. Collot, J. Gradinaru, N. Humbert, M. Skander, A. Zocchi, T. R. Ward, *J. Am. Chem. Soc.* **2003**, 125, 9030–9031.
- M. E. Wilson, G. M. Whitesides, *J. Am. Chem. Soc.* **1978**, 100, 306–307.
- K. Okrasa, R. J. Kazlauskas, *Chem. Eur. J.* **2006**, 12, 1587–1596.
- A. Fernandez-Gacio, A. Codina, J. Fastrez, O. Riant, P. Soumillion, *ChemBioChem* **2006**, 7, 1013–1016.
- T. Sugimoto, T. Kokubo, J. Miyazaki, S. Tanimoto, M. Okano, *J. Chem. Soc. Chem. Commun.* **1979**, 402–404.
- A. Mahammed, Z. Gross, *J. Am. Chem. Soc.* **2005**, 127, 2883–2887.
- J. R. Carey, S. K. Ma, T. D. Pfister, D. K. Garner, H. K. Kim, J. A. Abramite, Z. L. Wang, Z. J. Guo, Y. Lu, *J. Am. Chem. Soc.* **2004**, 126, 10812–10813.
- F. van de Velde, L. Könemann, F. van Rantwijk, R. A. Sheldon, *Chem. Commun.* **1998**, 1891–1892.
- M. T. Reetz, N. Jiao, *Angew. Chem.* **2006**, 118, 2476–2479; *Angew. Chem. Int. Ed.* **2006**, 45, 2416–2419.
- S. Colonna, A. Manfredi, R. Annunziata, *Tetrahedron Lett.* **1998**, 39, 3347–3350.
- R. S. Roy, B. Imperiali, *Protein Eng.* **1997**, 10, 691–698.
- J. Pierron, C. Malan, M. Creus, J. Gradinaru, I. Hafner, A. Ivanova, A. Sardo, T. R. Ward, *Angew. Chem.* **2008**, 120, 713–717; *Angew. Chem. Int. Ed.* **2008**, 47, 701–705.
- X. Y. Li, D. R. Liu, *J. Am. Chem. Soc.* **2003**, 125, 10188–10189.
- a) I. A. Kozlov, L. E. Orgel, P. E. Nielsen, *Angew. Chem.* **2000**, 112, 4462–4465; *Angew. Chem. Int. Ed.* **2000**, 39, 4292–4295; b) M. Bolli, R. Micura, A. Eschenmoser, *Chem. Biol.* **1997**, 4, 309–320.
- P. Ordoukhanian, G. F. Joyce, *J. Am. Chem. Soc.* **2002**, 124, 12499–12506.
- B. Seelig, S. Keiper, F. Stuhlmann, A. Jäschke, *Angew. Chem.* **2000**, 112, 4764–4768; *Angew. Chem. Int. Ed.* **2000**, 39, 4576–4579.
- G. Roelfes, B. L. Feringa, *Angew. Chem.* **2005**, 117, 3294–3296; *Angew. Chem. Int. Ed.* **2005**, 44, 3230–3232.
- G. Roelfes, A. J. Boersma, B. L. Feringa, *Chem. Commun.* **2006**, 635–637.
- A. J. Boersma, B. L. Feringa, G. Roelfes, *Org. Lett.* **2007**, 9, 3647–3650.
- A. J. Boersma, J. E. Klijn, B. L. Feringa, G. Roelfes, *J. Am. Chem. Soc.* **2008**, 130, 11783–11790.
- D. Coquière, B. L. Feringa, G. Roelfes, *Angew. Chem.* **2007**, 119, 9468–9471; *Angew. Chem. Int. Ed.* **2007**, 46, 9308–9311.
- A. J. Boersma, B. L. Feringa, G. Roelfes, *Angew. Chem.* **2009**, 121, 3396–3398; *Angew. Chem. Int. Ed.* **2009**, 48, 3346–3348.
- a) N. Shibata, H. Yasui, S. Nakamura, T. Toru, *Synlett* **2007**, 1153–1157; b) E. W. Dijk, B. L. Feringa, G. Roelfes, *Tetrahedron: Asymmetry* **2008**, 19, 2374–2377.
- a) T. N. Grossmann, A. Strohbach, O. Seitz, *ChemBioChem* **2008**, 9, 2185–2192; b) N. Sancho Oltra, G. Roelfes, *Chem. Commun.* **2008**, 6039–6041; c) Z. Tang, A. Marx, *Angew. Chem.* **2007**, 119, 7436–7439; *Angew. Chem. Int. Ed.* **2007**, 46, 7297–7300; d) L. Ropartz, N. J. Meeuwenoord, G. A. van der Marel, P. van Leeuwen, A. M. Z. Slawin, P. C. J. Kamer, *Chem. Commun.* **2007**, 1556–1558; e) Z. Tang, D. P. N. Gonçalves, M. Wieland, A. Marx, J. S. Hartig, *ChemBioChem* **2008**, 9, 1061–1064.
- a) S. Otto, J. Engberts, *J. Am. Chem. Soc.* **1999**, 121, 6798–6806; b) S. Otto, F. Bertoncin, J. B. F. N. J. Engberts, *J. Am. Chem. Soc.* **1996**, 118, 7702–7707; c) F. M. Menger, C. E. Portnoy, *J. Am. Chem. Soc.* **1967**, 89, 4698–4703.
- See the Supporting Information.
- F. Bailly, C. Bailly, M. J. Waring, J. P. Hénichart, *Biochem. Biophys. Res. Commun.* **1992**, 184, 930–937.
- C. Liu, Z. Jiang, Y. Zhang, Z. Wang, X. Zhang, F. Feng, S. Wang, *Langmuir* **2007**, 23, 9140–9142.
- J. Šebestík, I. Stibor, J. Hlaváček, *J. Pept. Sci.* **2006**, 12, 472–480.
- M. P. Sibi, Y. H. Yang, *Synlett* **2008**, 83–88.
- M. E. Quiroz-Castro, G. A. van Albada, I. Mutikainen, U. Turpeinen, J. Reedijk, *Inorg. Chim. Acta* **2000**, 297, 129–133.

- [41] R. Kannappan, S. Tanase, I. Mutikainen, U. Turpeinen, J. Reedijk, *Inorg. Chim. Acta* **2005**, 358, 383–388.
- [42] H. Kooijman, A. L. Spek, D. Rehorst, W. L. Driessen, J. Reedijk, *Acta Crystallogr. Sect. C* **1997**, 53, 1596–1598.
- [43] R. Paulini, K. Müller, F. Diederich, *Angew. Chem.* **2005**, 117, 1820–1839; *Angew. Chem. Int. Ed.* **2005**, 44, 1788–1805.
- [44] D. Fornasiero, T. Kurucsev, *Biophys. Chem.* **1985**, 23, 31–37.
- [45] M. Ardhammar, T. Kurucsev, B. Nordén in *Circular Dichroism: Principles and Applications*, 2nd. ed. (Eds.: N. Berova, K. Nakanishi, W. R. Woody), Wiley-VCH, Weinheim, **2000**, Chapter 26.

Received: February 19, 2009

Revised: April 22, 2009

Published online: July 3, 2009